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Evaluation of Preservation Methods for Cadavers: Hard Tissue Investigation

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Introduction

Nearly as long as bones have been tested, the effects of preservation on their material properties have been studied. A previous review of the literature on the material properties of preserved hard tissues, however, showed varied and inconclusive results (Crandall, 1991). The only consistent findings are that freezing and thawing appears to maintain the properties of hard tissue. Meanwhile, the results for the material properties of embalmed bone specimens vary between no change and statistically significant alterations relative to the fresh condition. These variations are frequently attributed to the lack of standardized test procedures and embalming preparations. The research presented in this paper maintains consistent procedures and examines a wide variety of commercial and custom embalming techniques. The research is part of an on-going project to develop preservation techniques for cadavers that provide tissue biofidelity, maintain preservation quality, and control the risk of infectious disease.

Preservation Methods

Embalming, freezing, and refrigeration were used in this study to preserve hard tissue specimens. Refrigeration is the most common form of short term storage for biological materials. In this research, refrigeration maintained the hard tissue specimens at +2°C to +5°C. To prevent dehydration, the samples were wrapped in gauze soaked in saline. For long-term storage, freezing is the most common method of preservation for tissues used in biomechanical testing. The most common and most detrimental side-effect of the frozen tissues is "freezer burn" and considerable attention must be given to packaging and to preparing the tissues in order to prevent dehydration. In this research, freezing maintained the hard tissue specimens at -70°C.

In this study, immersion of tissue specimens in embalming solutions was also used to preserve the hard tissue prior to testing. Tissue preservation through embalming is accomplished through a chemical "fixation" or coagulation of the proteins within the tissue cells. Embalming chemicals produce cross-linkages between adjacent proteins that are not normally present in the living tissue. The result is a complex latticework of inert, firm material that is no longer a suitable medium for bacteria. In addition, these inactive stabilized proteins are extremely resilient to the body cell's autolytic enzymes. A thorough review of embalming theory and procedures can be found in Crandall (1991). Today, a plethora of commercial embalming fluids are available for cadaver preservation. Formulations range from waterless to non-toxic fluids for which the principal components include everything from formalin to lanolin. Most embalming solutions formaldehyde or glutaraldehyde as the basis of the fluid. Recent state and federal Occupational Safety and Health Administrations (OSHA Va., 1988; OSHA, 1990), however, have imposed exposure limits for formaldehyde. In response to the new legislation, chemical companies have begun to produce non-aldehyde solutions that appeal to embalmers not wanting to deal with risk of exposure and the expense of complying with the new formaldehyde standards. Although, the resulting fluids are classified as non-toxic by the Environmental Protection Agency (EPA), they purport levels of preservation and disinfection comparable to traditional embalming fluids.

Chemists from the major embalming fluid manufacturers were contacted at the start of this research and informed of this project's requirements for biofidelity and tissue preservation. A brief description of the four embalming fluids meeting the selection criteria is provided.

Biofidelity Fluid (University of Virginia; Charlottesville, VA)

The Biofidelity fluid is based on a European anatomical embalming solution referred to as the Winckler fluid (Winckler, 1974). The formaldehyde based Winckler fluid was originally developed to preserve cadavers for anatomical studies but has since been used in biomechanical studies by the INRETS laboratory in Lyon, France. The University of Virginia (UVA) modified the Winckler fluid by reducing the levels of formalin, using distilled water, adding water conditioners and pH buffers, and eliminating some agents considered to be extraneous (Crandall, 1994). The resulting formulation, herein referred to as the Biofidelity fluid, has been used at UVA to embalm successfully more than 40 cadavers for biomechanical studies.

Michigan Anatomical Fluid (Dodge Chemical Company; Boston, MA)

The Michigan Anatomical fluid is a waterless solution composed primarily of formaldehyde (3%) with significant quantities of methanol (0.8%) and isopropanol (6.0%). Based on recommendations from the manufacturer, the Michigan Anatomical fluid was used in this study because it has reportedly maintained tissue compliance and preservation in medical school anatomical cadavers.

PLX (Champion Chemical Company; Springfield, IL)

PLX is a glutaraldehyde based fluid that contains lanolin to retain tissue moisture and flexibility. Although glutaraldehyde (6%) is the primary preservative and disinfectant, PLX also contains a significant amount of formaldehyde (4%) and methanol (11%). The PLX solution was chosen for this study because it was one of the few commercially available glutaraldehyde solutions.

STF (Streck Laboratories; Omaha, NE)

The STF fluid is a non-toxic solution currently used to prepare tissues for histology studies but intended to be used for embalming in the future. The STF fluid contains a combination of organic salts that act with a fixative agent to preserve the tissue. The fixative agent is a urea compound that releases only after entering the body. Since the solution is not hazardous during the material handling or injection procedures, the fluid is considered non-toxic and is exempt from EPA and OSHA regulations. The manufacturer claims that STF will preserve the tissues as well as aldehydes while simultaneously producing softer, more pliable tissues.

Materials

One hundred and fifty (150) rib samples from thirteen cows were acquired from a local slaughterhouse. All samples were obtained less than 24 hours post-mortem and were refrigerated for the duration of the preparation and the preservation procedures. After separating and cutting them to a test length of approximately 30 cm, the bovine ribs from each animal were tagged with the appropriate steer number. Although the surrounding musculature was removed from the specimens, the fibrous membrane that forms the covering of bones (i.e., the periosteum) was left in-tact.

The ribs of both humans and cows can vary significantly in size, orientation, and taper, as they progress from the top to the bottom of the thorax. To test the significance of their anatomical position, adjacent ribs were grouped into statistical blocks according to rib number. No distinctions were made between ribs removed from the left and the right sides. Because of their curvature and taper, the top ribs (rib 1 and rib 2) and the bottom rib (rib 14) were not used for mechanical testing in this study.

In addition to the effects of the preservation fluid on bone, the temporal effects of storage were investigated. For a given rib number, the ribs from all cows were pooled and randomly sorted into a test matrix of times and preservation fluids. Rib samples were refrigerated, frozen, or embalmed for various lengths of time (2 days, 5 days, 12 days, 26 days, 40 days) before testing. A rib from each anatomical position (i.e., rib number) was tested with every storage method at every time interval. The samples were placed in polyethylene containers and either immersed in an embalming fluid or placed in the freezer/refrigerator. To ensure a humid environment, the frozen and the refrigerated samples were wrapped in gauze soaked with saline before they were placed in the plastic bags.

Those ribs that were not tested (i.e., ribs number 1, 2, and 14) were used in ashing tests. The mineral content results from these tests estimated the cow's skeletal integrity and physiologic condition. The resulting ash ratio was later used in the regression analysis to account for inherent differences among the cows.

Test Methodology

Three-point bending tests were used to compare the fresh and the preserved rib material properties. The methodology for rib bending tests was well documented (Granik and Stein, 1972) and the test samples required no machining of bone. The ribs were situated in a specially designed three-point bend fixture with a span of approximately 16 cm. The exact dimensions of the fixture were adjusted to ensure that the most lateral region of the rib shaft (i.e., the straightest portion) was contacted. Positioning the rib in this manner minimized the contributions of rib taper and curvature.

All experiments were performed using a Tinius Olsen Universal Test Machine (Model 30,000 lb. (134 kN) LoCap). To provide for superior resolution in the range of anticipated forces, a calibrated 1,000 lb. (4.45 kN) load cell was used. The 500 lb. (2.23 kN) range capacity used in testing had an accuracy of +/- 0.5 lb. (2.3 N) and a resolution of 0.005 lb. (.023 N). Computer control and data acquisition were operated through the DS-50 Tinius Olsen Control Module linked with a bi-directional RS-232 interface to a personal computer. All rib specimens were tested at a position control rate of 2.5 mm/min. The three-point bend test fixture applied the quasi-static load at the midspan of the rib. The midspan deflection and the applied forces were respectively recorded by the cross-head's linear variable differential transducer (LVDT) and load cell. All loading was performed at the constant rate until failure occurred. The prevalence of "green-stick" fractures (i.e., fractures of the bone that did not produce separation of the bone pieces adjacent to the break) necessitated that failure be designated by a predetermined criterion. Thus, failure was identified at the point in which a drop in load greater than or equal to 10% of the previous maximum load occurred.

Computed tomography (CT) proved to be the most efficient, convenient, and least expensive method of determining the requisite geometric properties of the ribs. Because it can distinguish minute differences in the density of a material, CT was able to discriminate between the cortical and the cancellous bone in an effective manner. Cross-sections were imaged using 0.5 mm contiguous slices along the shaft of the rib. A single cross-section, in close proximity to the break, was used to calculate the cross-sectional properties for the entire rib segment. Although only the cross-sectional area was required in the analysis of the bend tests, the taper and the curvature were also determined from the CT images.

The digitized cross-sections were represented by computer pixels with each pixel representing a finite area (dA). After digitization, a simple determined the geometric properties of the rib cross-sections from the images. The total cross-sectional area (A) was equal to the sum of all n pixels. The maximum width of each rib, measured with a vernier caliper, served as the generic scaling factor. The cross-sectional properties, including the area and the moments of inertia, were calculated.

Analysis

The force-deflection curves obtained from the rib testing were converted to stress-strain curves using elastic beam theory. The elastic modulus, ultimate strain, ultimate stress, and the yield stress were determined for all of the rib samples. Next, a linear statistical model was created for each material property as a function of the experiment's independent categorical variables (i.e., cow, rib pair, and preservation fluid) and of the experiment's continuous variables (i.e., time and ash content). The statistical models were the following:

Model (A): Ultimate Stress $= \mu + \underline{\beta}$ [Cow : Rib : Fluid : Cow*Fluid : Cow*Time : Fluid*Time : Time : Ash]^T + Error

Model (B): Ultimate Strain $= \mu + \underline{\beta}$ [Cow : Rib : Fluid : Cow*Fluid : Cow*Time : Fluid*Time : Time : Ash]^T + Error

Model (C): Elastic Modulus $= \mu + \underline{\beta}$ [Cow : Rib : Fluid : Cow*Fluid : Cow*Time : Fluid*Time : Time : Ash]^T + Error

Model (D): Yield Stress $= \mu + \underline{\beta}$ [Cow : Rib : Fluid : Cow*Fluid : Cow*Time : Fluid*Time: Time : Ash]^T + Error

The Cow, Rib, Fluid, Time, and Ash variables referred to the steer, the rib number, the preservation method, the time of storage, and the mineral content respectively. For each model, the mean, μ, represents the average response parameter for all 120 ribs. The model's independent categorical variables (i.e., cow, rib number, and preservation fluid) are vectors containing the appropriate number of constants. The vectors with multiplicative terms signify interactions of the categorical variables with the continuous variables. Finally, the matrix $\underline{\beta}$ contains the coefficient estimators weighting each of the input variables. Once determined, the $\underline{\beta}$ matrix can be used to predict changes in the response parameters for incremental changes in the independent variables.

To determine whether or not the material properties of the ribs differed among the preservation groups, the rib data were subjected to a multi-variable analysis of variance (ANOVA). ANOVA analysis determined if the mean for a given sample was truly different than the underlying population mean or merely appeared similar due to chance fluctuations. The F test, a common statistical test, further examined the ratio of these sample variances (i.e., variance between the sample means) to the overall pooled variance (i.e., variance within the sample groups). The final result was a probability value that quantifies the credibility that no differences exist among the different sample groups (i.e., the null hypothesis. Since the main factor of interest was the Fluid type, the Duncan's Multiple Range test at the 95% confidence level was performed for each material property.

Elastic Modulus - Model (C)

The elastic moduli data for the bovine ribs (Model C) were analyzed using ANOVA and the F test. The F test found all independent variables and interactions to be statistically significant at the 95% level of confidence. In addition, the subsequent Duncan's multiple range test concluded that the elastic moduli for all fluids were statistically distinct. Each fluid group comprised its own grouping and all fluids altered the elastic modulus based on the fresh condition (Table 1).

Preservation Fluid	Elastic Moduli (GPa)
Refrigerated Environment	11.7
Biofidelity Fluid	10.9
Michigan Anatomical Fluid	10.7
STF Fluid	10.0
Frozen Environment	9.2
PLX Fluid	8.8

Table 1. Elastic moduli of preserved ribs.

Yield Stress - Model (D)

The analysis of the yield stress data demonstrated that each independent variable and each interaction in Model D was statistically significant at the 95% confidence level. Furthermore, the Duncan's multiple range test concluded that the yield stress values for each fluid group were statistically distinct. Including the frozen storage method, no preservation technique successfully retained the properties of the ribs in the fresh condition (Table 2).

Preservation Fluid	Mean Yield Stress (MPa)
Biofidelity Fluid	282
Refrigerated Environment	281
Michigan Anatomical Fluid	271
STF Fluid	266
Frozen Environment	236
PLX Fluid	225

Table 2. Yield stresses of preserved ribs.

Ultimate Stress - Model (A)

ANOVA analysis with the ultimate stress model (Model A) determined that all variables and interactions were significant. Thus, the associated null hypotheses could all be rejected at the 95% level of confidence. The Duncan's multiple range test also concluded that the ultimate stress values for different Fluids were statistically significant (Table 3).

Preservation Fluid	Mean Ultimate Stress (MPa)
Michigan Anatomical Fluid	340
Refrigerated Environment	336
Biofidelity Fluid	334
STF Fluid	308
Frozen Environment	282
PLX Fluid	276

Table 3. Ultimate stresses of preserved ribs.

Ultimate Strain - Model (B)

According to the ANOVA analysis, all factors (i.e., variables) were found to be statistically significant at the 95% level of confidence. However, the Duncan's test concluded that statistically significant differences do not exist among the fluid preparations (Table 4). Since the Duncan's multiple range test could not distinguish statistical significance among the fluids for ultimate strain, however, the ultimate strain data is included only in an effort to distinguish a trend. However, the reader should keep in mind that any percent changes in ultimate strain over the fresh condition could be due to spurious resulting from the large standard deviations of the data. In other words, the null hypothesis

that the preservation methods do not change the mean ultimate strains of fresh tissue cannot be rejected.

Preservation Fluid	Mean Ultimate Strain (%)
Frozen Environment	6.125
STF Fluid	5.211
Refrigerated Environment	4.941
Michigan Anatomical Fluid	4.857
PLX Fluid	4.800
Biofidelity Fluid	4.368

Table 4. Mean ultimate stress values for preserved ribs.

Since the preservation groups were significant for all response parameters except strain, they can be compared on a percentage basis from the fresh condition. Investigating the percent differences among the preservation groups, the fresh condition was used as the control group (Table 5).

Fluid	Elastic Modulus	Yield Stress	Ultimate Stress	Ultimate Strain
Michigan Anatomical Fluid	-9.8 %	-3.5 %	+1.4 %	-1.7 %
PLX Fluid	-26.2 %	-20.0 %	-17.9 %	-2.9 %
STF Fluid	-15.6 %	-5.5 %	-8.2 %	+5.5 %
Biofidelity Fluid	-8.0 %	+0.2 %	-0.7 %	-11.6 %
Frozen	-22.4 %	-20.0 %	-16.0 %	+24.0 %

Table 5. Mechanical Properties of preserved ribs relative to the fresh condition.

Since the freezing and thawing process has traditionally exhibited no change in the material properties of hard tissue, the frozen test condition was used as an alternative control group (Table 6).

Fluid	Elastic Modulus	Yield Stress	Ultimate Stress	Ultimate Strain
Michigan Anatomical Fluid	+8.7 %	+15.0 %	+20.7 %	-20.7 %
PLX Fluid	-4.9 %	-4.7 %	-2.2 %	-21.6 %
STF Fluid	+8.7 %	+12.7 %	+9.3 %	-14.9 %
Biofidelity Fluid	+18.5 %	+19.5 %	+18.3 %	-28.7 %
Refrigerated	+29.0 %	+19.2 %	+19.1 %	-19.3 %

Table 6. Mechanical Properties of preserved ribs relative to the frozen condition.

Contrary to the results presented in the literature search, the fresh and frozen bone samples showed markedly different material properties.

Discussion and Conclusions

The linear response of the rib's force-deformation histories lends credence to this paper's analysis of the rib response using elastic beam theory. Inaccuracies, however, can be incurred in bending tests due to specimen misalignment, friction at the load points, imprecise strain measurement, inadequate length-to-cross-section ratio, specimen curvature and taper, and elastic-plastic deformation. Small length-to-cross-section ratios can also create biaxial stress distributions. In addition, elastic-plastic deformations occur when the yield stress is exceeded in the outermost fibers of the test specimen. Fortunately, the

differences in response among the preservation groups were sufficiently large to ignore contributions from any violations of the testing assumptions. Consistent failure of the bones on the bottom of the specimens indicated that the bones failed in tension and not in shear.

Upon first inspection of the mechanical property data in Table 6, the results for the Biofidelity fluid appear to approximate most closely those of the fresh tissue. Table 6 shows minimal changes in the yield stress (0.2%) and the ultimate stress (0.7%). In addition, the data demonstrate slightly greater alterations in the elastic modulus (8%) and the ultimate strain (11.6%). Although the change in strain is quite large, it is not statistically significant based on the results of the Duncan's multiple range test.

The most surprising result from the analysis of the material property data was the significant decrease in material properties for the frozen specimens relative to the fresh condition. The reader will recall that the freezing process has been extensively validated as a preservation technique and does not typically alter the material properties of hard tissue. Investigation of this study's data, however, indicated that the relative change in frozen samples might actually be due to an increase in the material properties of the refrigerated samples over time. As explained in the preparation section, both the refrigerated and frozen specimens were wrapped in gauze soaked with saline and placed in cold storage. Collectively, all frozen or refrigerated specimens were grouped in a single plastic bag that was closed but not vacuum sealed. One possible explanation for the relative difference in material properties between the fresh and frozen ribs is that the ribs in the cooler dehydrated while those in the freezer did not. The cold air in both the cooler and freezer is extremely dry and contains limited moisture. It is quite possible that the gauze and eventually the refrigerated ribs partially dehydrated because of their contact with the cold, dry air. Meanwhile, the gauze surrounding the frozen ribs would have immediately frozen and would have created a moisture barrier that prevented further dehydration. Upon thawing, the ribs would once again be stored in a humid environment and absorb fluid from the surrounding fluid. Thus, the possibility that the refrigerated bones may have dehydrated while the frozen ribs may have remained moist prompted additional literature searches and further analysis of the data.

The role of water in determining bone material properties is vital. Water accounts for more than 25% of the wet weight of cortical bone (Viano, 1986). When bone is dried, only a fraction of the water is bound sufficiently tight between the organic matrix and mineral salts to remain. Because early research attempts to produce machined bone samples frequently resulted in dehydration of the samples, the effects of drying on the material properties of bone have been extensively studied. Furthermore, all investigators who have studied the drying of bone have found that the tensile and compressive strengths, the modulus of elasticity, and the hardness of bone are increased significantly

If the frozen rib material properties are assumed to represent the fresh, unaltered condition, Table 6 demonstrates a 20% to 30% increase in the refrigerated tissue stresses and moduli. Furthermore, the refrigerated ribs exhibited nearly a 20% decline in strain relative to the frozen ribs. All of these occurrences, as well as the associated magnitudes of change, are consistent with those identified by others researching the drying of bone.

To investigate the extent to which the material properties were altered by drying, temporal changes in the rib properties were studied for all four response parameter models. The estimated regression coefficients for time were extrapolated from the β matrix (Table 7). For all responses except ultimate strain, time proved a significant variable in two fluid groups: the glutaraldehyde (PLX) and the refrigerated fresh. In addition, all the estimated coefficients were positive, meaning that the ultimate stress, elastic modulus, and yield stress for the two groups increased over time. Since most of the response groups were weighted by the inverse of their variance, interpretation of the estimators and the regression model cannot be direct. Although the intercept of the linear regression is unknown, relative increases over time can be estimated. Since the elastic moduli for the

refrigerated fresh condition increased 145 MPa per day, a total increase of nearly 5.52 GPa can be estimated for the maximum period of testing. Clearly, the magnitude of these estimates reinforces the hypothesis that the refrigerated ribs dehydrated and resulted in significant changes in material properties over time. Consequently, the frozen preservation group was henceforth considered to be the control group.

The level of statistical significance in Table 7 requires further elucidation. The typical coefficient for the 95% level of confidence is 0.05. Although the p-values of the stress data are below these values (i.e., the null hypothesis that time does not affect the stress can be rejected), the elastic moduli exceed the p-value corresponding to the 95% level of confidence. The temporal changes in the elastic moduli, however, are significant at the 90% level of confidence (i.e., p-value > 0.1). Thus, the moduli results should be considered "marginally significant". Although the absolute level of change with time is not essential, the elastic moduli decidedly indicate an increasing trend with time of preservation for the fresh and PLX fluid (i.e., glutaraldehyde based) mediums.

Response	Fluid	Time Estimate	Standard Deviation	p-value
Ultimate Stress	PLX	603.4	248.0	.07
(weighted)	Refrigerated Fresh	410.2	114.3	.01
Ultimate Strain	None			
(unweighted)				
Elastic Modulus	PLX	25161	12006	.09
(unweighted)	Refrigerated Fresh	21069	10489	.09
Yield Stress	PLX	756.2	275.4	.04
(weighted)	Refrigerated Fresh	238.3	73.16	.02

Table 7. Time regression coefficients for rib material properties.

Although ribs preserved with the formaldehyde solutions (i.e., Biofidelity and Michigan Anatomical fluids) showed significant increases in material properties relative to the frozen ribs (ranging from 9% to 20%) , ribs preserved with PLX fluid showed decreases in all response parameters except ultimate strain (ranging from -2% to -5%). Two possible explanations exist for these decreases in strength with the dialdehyde preservation process. First, the glutaraldehyde in the PLX fluid may not have sufficiently preserved the tissue when compared to the those tissues preserved with formaldehyde. Subsequent to the breakdown of the tissue at the microscopic level, rib strength at the macroscopic level would have been compromised. Second, the material properties of the ribs preserved with the glutaraldehyde based PLX fluid may not have reached their maximum values. Recall that the material properties of these ribs showed positive and increasing coefficients over time.

Another surprising result from this testing is the fact that the amount of formaldehyde in an embalming solution is not the only factor responsible for increases in strength. Experience in embalming bodies with both the Michigan Anatomical fluid a .1 the Biofidelity embalming solution has shown qualitatively much stiffer tissues in bodies embalmed with the former (Crandall, 1994). Although both solutions are formaldeh de based, the Michigan Anatomical solution contains a much larger percentage of formaldehyde (3.0% versus 0.56%). Embalming theory would predict greater protein cross-linkage, and subsequently greater tissue rigidity, for higher levels of formaldehyde. Thus, the ribs preserved with the Michigan Anatomical fluid should exhibit greater moduli than those preserved with the Biofidelity embalming fluid.

A secondary mechanism other than protein cross-linking must be causing the tissue's stiffness. Although, it is possible that the higher concentrations of formaldehyde could have fixed the outer fibers of the ribs and not permitted fluid to penetrate throughout the entire volume, the small size of the specimen relative to an entire body and the lengthy time of immersion make this hypothesis improbable.

The percentage of hydrogen ions in solution and the temperature of the tissue can also influence the degree of preservation and penetration by the aldehydes. Both aldehyde solutions, however, contained pH buffers and were maintained at constant temperatures to control the reaction rate and to limit the extent of tissue fixation. Perhaps the only conclusion that can be drawn from this data is that both formaldehyde solutions significantly altered the hard tissue material properties. Although these results coincide with those of Carothers et al. (1949) and Evans (1973) , they differ from those of McElhaney et al. (1964).

Since both belong to the aldehyde family of chemicals, the fact that glutaraldehyde (PLX Fluid) showed decreases while formaldehyde (Biofidelity and Michigan Anatomical Fluids) demonstrated increases in the material properties is interesting. Formaldehyde is often combined with glutaraldehyde in embalming fluids for stability. The rib testing results suggest that a combination of these two aldehydes could theoretically obtain material properties nearly identical to those of fresh hard tissue. A formulation of this type would combine the excellent germicidal qualities of glutaraldehyde with the superior fixative properties of formaldehyde.

It is particularly interesting to observe the changes in the properties of the ribs preserved with the non-toxic formulation (STF). If the frozen specimens are taken as the standard, the non-toxic formulation exhibited the smallest increase in material properties. The elastic moduli (8.7%), the yield stress (12.7%), and the ultimate stress (9.3%) were small relative to the 15% to 20% changes found in the formaldehyde and refrigerated rib samples.

The cow and ash content were both significant variables in the analysis of the material properties. In every model, these dependent variables and their associated with other variables had a marked influence on the results (i.e., p-values > .0001). This result emphasizes the fact that mechanical testing of biological specimens must combine estimates of both the ante-mortem physiologic condition and the post-mortem storage conditions when determining material properties.

Acknowledgments

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DISCUSSION

PAPER: **Evaluation of Preservation Methods for Cadavers**

PRESENTER: Jeff R. Crandall, University of Virginia

Q: Guy Nusholtz, Chrysler Corporation
What was your source for radiation? Was that Cobalt 60?

A: Yes.

Q: And you chose the amount of rads by the amount of time it was exposed?

A: It was at a constant rate. I believe it's .125 megarads per hour.

Q: So you sort of wheeled the body into the room.

A: These were just specimens.

Q: Then you raised the source and, at a given time, then lowered it.

A: Exactly.

Q: Did you try diluting the glutaraldehyde?

A: What I did that I probably should have said in the beginning, was I contacted all the chemical companies that produce embalming fluids and told them, this is what we want to do. We want to produce a biofidelic specimen and to maintain the properties of fresh tissue. So I said, give me your solution, give me your best fluid. Tell me how to use it and we'll see what we get for results. I went by the manufacturer's recommendations on all the fluids.

Q: OK, so they sent you the fluid and then you used it as they said. So you didn't work with them?

A: I worked with them. Yes, I did. I told them what we wanted to do and they modified some of the solutions from their standard anatomical embalming and they also modified the procedure. Some had me dilute the solution. Some asked me what I was using for formaldehyde levels. So I actually spoke with a chemist at every one of the companies we obtained them through.

Q: When I worked with glutaraldehyde, I had a lot of problems trying to change the concentrations and everything. I don't know if you ran into the same difficulties or not.

A: Again, we all used one concentration.

Q: OK, could you sort of explain what you meant when you were talking about the tissue degradation, the histology? What you are saying is that the glutaraldehyde maintained the

physical properties, but yet, at the cellular level, it didn't sort of maintain.

A: Exactly. The histologic specimens were right in terms of the degree of autolysis. Autolysis is breakdown of the tissue as recorded as loss of cellular detail. There is something called sloughing of cells where epithelial cells start to pull away. These sort of parameters were evaluated by a pathologist and assigned a subjective rating of zero to five, none to severe.

Q: Somehow both properties stay the same and yet the microproperties change?

A: Exactly.

Q: That's an interesting contradiction. OK, thank you.

Q: Frank Pintar, Medical College of Wisconsin
It's a nice study, Jeff. What was the freezing temperature that you used?

A: We froze at -73 degrees Celsius, and all the specimens were thawed at room temperature: +75 degrees Fahrenheit or 20 degrees Celsius.

Q: Wait a minute. You used -20 degrees Celsius for the freezing?

A: -73 degrees Celsius.

Q: Oh, OK. So it was a deep freeze.

A: Yes.

Q: OK. I was wondering, if it's really a very deep freeze at -70, you're really supposed to not get any degradation of tissue.

A: Yes. I think what you are referring to is, if you freeze very rapidly, you are supposed to get smaller cellular ice crystals forming than if you freeze more slowly. What you'd ideally like to do is freeze at as cold a temperature as possible, and we try to do that.

Q: Just a congratulations on a very large body of work. You presented hard tissue and then soft tissue being structures that are largely cellular with a little bit of parenchymal structures. What's your experience been with the other class of tissues, tendon, ligament?

A: We're just starting a ligamentous study. Since we're doing the lower extremity talk, the lower extremity project which I gave a talk on earlier, we're trying to characterize ligament properties with preservation. We're just in the early stages of that. Maybe next year I can come back and tell you where we stand.

Q: I look forward to that.

A: Thank you.